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ORIGINAL PAPER

Linear compartmental systems. IV. A software, under MS-Windows, for obtaining the instantaneous species concentrations in enzyme systems

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Abstract Software application is implemented in this work to take full advantage of the characteristics of current operating systems and to provide the optimized symbolic kinetic equations for both enzyme and ligand species involved in enzyme reac-

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tions. This software, called SKEE-w2013, is implemented using C# language and runs under all operating systems from Windows XP up to Windows 8. It is applicable to any enzyme reaction mechanism that fits the general reaction scheme proposed previously by our group. It can be downloaded, free of charge, from http://oretano.iele-ab.uclm.es/~BioChem-mg/software.php. Besides the optimized equations, the software can provide non-optimized equations, so that the user can compare the advantage of using optimized equations rather than the non-optimized ones, whenever they do not coincide. Moreover, the software circumvents the limitations of other existing previous software tools implemented with what are nowadays obsolete programming languages and that, moreover, are limited to non-optimized kinetic equations.

Keywords Enzyme system · Kinetics · Enzyme species · Ligand species · Symbolic equation · Software

1 Introduction

In paper III of this series [1], symbolic kinetic equations, called optimized equations, were obtained. These equations furnish the instantaneous concentration of any enzyme or ligand species involved in enzyme reactions, whose mechanism fits the general model described therein. In previous contributions, kinetic equations were obtained for enzyme reaction mechanisms fitting [2–4] or not fitting [5–10] this general model. Also, symbolic kinetic equations were obtained for enzyme reactions fitting this same general model. Nevertheless, these equations were not optimized because they contained all the kinetic parameters and initial concentrations, even though some of them would have no influence in the instantaneous concentration under study, as in the examples indicated in [1]. Varon et al. [2] and Garcia-Meseguer et al. [3,4] provided algorithms to facilitate obtaining the coefficients involved in the equations obtained by these same authors, avoiding expansion of determinants as well as matrix inversions. This recurrent and systematic way of obtaining these coefficients allowed the implementation of the software, all of them in BASIC or Visual BASIC languages for MS-DOS operating systems, which facilitated the obtaining of the equations [11–17]. The software programs mentioned above have two important limitations: (1) they refer to the non-optimized, general equation, with the attendant disadvantages illustrated with examples in [1]; and (2) the programming languages used in all of them are nowadays obsolete, meaning slow processing times, reaction mechanisms that cannot involve a high number of enzyme species and, finally, the fact that the software does not run under current operating systems, anyway.

The aim of the present contribution is to circumvent the above two limitations by implementing a specific software application directly, providing the optimized equations corresponding to any enzyme reaction fitting the general model described in [1], irrespective of the number of enzyme species present at the outset of the reaction. The software implemented here takes full advantage of the performance of modern C# language and the characteristics of current operating systems. The results obtained in [1,18] are taken as the starting point to fulfill the above aims.

The optimized equations derived in [1] have the advantages over the non-optimized ones that they are given in the most possible simplified form in a double sense. (1) the

only kinetic parameters involved in them are those, which influence the instantaneous concentration of the enzyme or ligand species under study—initial concentrations and exponential terms—and (2) the arguments of the involved exponential terms, *i.e.* parameters λ_h (h taking one or more of the values 1,2,…u) are obtained as the roots of irreducible polynomials. Nevertheless, the equations for the instantaneous concentrations of the ligand species can be expressed in a more compact form which is more suitable for their computerized treatment. Therefore, the first step prior to implementation of the software is to adapt the optimized equations for the ligand species to facilitate their computerized derivation.

2 Adaptation of the optimized equations for the ligand species to facilitate their computerized treatment

2.1 Notation and definitions

In the following, the terms enzyme species and compartment are used interchangeably because the set of enzyme species involved in the reaction mechanism is handled as a compartmental linear system. Likewise and due to this, the terms enzyme system, compartmental system or merely system are equivalents. To render the optimized equations in a form that improves their computerized treatment it is convenient to use a particular notation, as well as certain definitions. Many of these notations and definitions were already introduced in the previous papers [1,18,19] of this series and, therefore only mention the most relevant ones. The reference to any equation of papers I, II or III are given as Eq. (paper number-equation number), e.g. Eq. (I-4), Eq. (II-3), Eq. (III-2), etc. Moreover, it is necessary to state some new notations and definitions. Hence, we indicate them:

n Number of enzyme species involved in the mechanism of the enzyme reaction.
g Number of ligand species involved in the mechanism of the enzyme reaction.

Initial directed graph Directed graph corresponding to the compartmental system that is equivalent to the enzyme system under study.

$X_1, X_2,\ldots, X_n$ The enzyme species in the initial directed graph.
Ω Set of enzyme species present at onset of the reaction.

Initial condensation diagram Condensation diagram, by classes, corresponding to the initial directed graph.

δ	Number of classes in the initial condensation diagram.
$C_1, C_2, \ldots, C_\delta$	The classes in the condensation diagram.
Φ_c	Set of the classes of the condensation diagram, $\Phi_c = \{C_1, C_2,\ldots, C_\delta\}$
ρ	Set of subindices in the notation of the classes, *i.e.* $\rho = \{1,2,\ldots,\delta\}$
s	Subindex assigned to the ligand species Y_s ($s = 1, 2, \ldots, g$)
L	Set of enzyme species, denoted as $X_{subindex}$, from which Y_s is released and/or to which Y_s binds.
l	Set of subindices in the notation of the enzyme species that belong to **L**.
θ	Set of enzyme species that are, simultaneously, successors of those belonging to set Ω and precursors of enzyme species of set **L**.

$\boldsymbol{E}_s$	Set of classes of the initial condensation diagram to which the enzyme species of setθ belong to. In any case, $E_s \subset \Phi_c$ is achieved. In [18], we defined the set $\boldsymbol{E}_i$ and some expressions related to it. These expressions and their meaning can be extrapolated to set $\boldsymbol{E}_s$. This set $\boldsymbol{E}_s$ is essential in order to obtain the optimized kinetic equations of Y_s because all involved $K_{i,j}$ are those such as $X_i \in E_s$.
$\rho(s)$	Set of subindices in the notation of the classes that belong to set $\boldsymbol{E}_s$. Obviously, $\rho(s) \subset \rho$.
$n(s)$	Number of enzyme species of **set** θ. This number coincides with the number of enzyme species belong to all classes of set $\boldsymbol{E}_s$.
$c(s)$	Number of final classes belong to set $\boldsymbol{E}_s$.
$u(s)$	$u(s) = n(s) - c(s)$
$\boldsymbol{z}(s)$	$z(s) = \bigcup_{i \in l} z(i)$
$\omega(s)$	Set of subindices in the notation of enzyme species belong to set Ω which have influence on $[Y_s]$.
$D(\lambda)$	Characteristic polynomial of $\boldsymbol{K}$.
$\lambda_h\ [h \epsilon\ \boldsymbol{z}(s)]$	Roots of polynomial $T_{E_s}(\lambda)$.

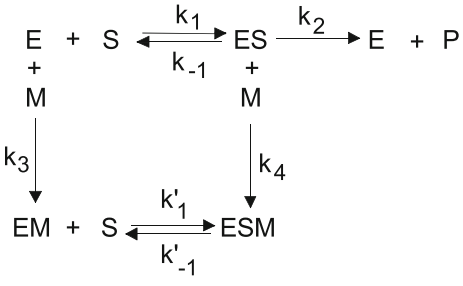
2.1.1 Examples of the notation and definitions above

For better understanding, we use example 2 of [1] and maintain its numbering herein. The corresponding reaction mechanism of this example is shown in Scheme 1. The directed graph and condensation diagram are shown in Fig. 1. The corresponding notation and definitions are shown in Table 1.

2.2 Alternative expressions of the optimized equations for the ligand species adapted for their computerized handling

Optimized Eqs. (III-3)-(III-5) for enzyme species are in such form that they are already appropriate for the implementation of a software to obtain them Thus, they do not require any subsequent adaptation.

Optimized Eqs. (III-7)-(III-9) and (III-11) for the instantaneous concentration of any of the ligand species, Y_s ($s = 1, 2, \ldots, g$) involved in the reaction mechanisms are given in a form that clearly distinguishes the separate contribution to $[Y_s]$ of any



Scheme 1 Model of an enzyme reaction consisting of the general Botts and Morales [22] modifier mechanism in which the modifier is irreversible and the product is only released from ES.

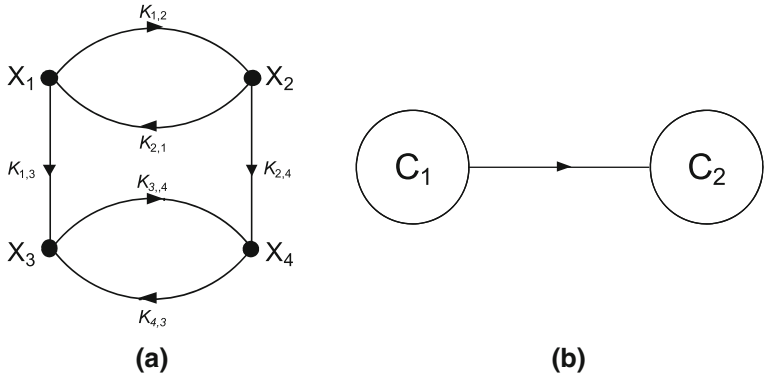


Fig. 1 **a** Directed graph of the enzyme reaction scheme 1. X_1, X_2, X_3 and X_4 denote the compartments corresponding to the enzyme species E, ES, EM and ESM, respectively. $K_{1,2}$, $K_{2,1}$, $K_{3,4}$ and $K_{4,3}$ are the fractional transfer coefficients. **b** Condensation diagram arising from the directed graph in (**a**). The classes are: $C_1 = \{X_1, X_2\}$ and $C_2 = \{X_3, X_4\}$. C_1 is an initial class and C_2 is a final one

of the enzyme species from which Y_s is released or with which Y_s combines. These forms of the optimized equations for Y_s are adequate and very useful for providing a comprehensive insight into the partial contribution to $[Y_s]$ of each of the enzyme species involved. Nevertheless, in order to simplify their computerized implementation in computer software, it is more convenient to express them in a more compact and suitable form. It can be shown that Eqs. (III-7)-(III-9) and (III-11) can be expressed as:

$$[Y_s] - [Y_s]_0 = \beta_s + \alpha_s t + \sum_{h \in z(s)} \gamma_{s,h} e^{\lambda_h t} \; (s = 1, 2, \ldots, g) \tag{1}$$

$$\alpha_s = \frac{\sum\limits_{(i,j)} \left\{ K_{j,i} \sum\limits_{k \in \omega(s)} (f_{k,j})_{u(s)}(E_s)[X_k]_0 - K_{i,j} \sum\limits_{k \in \omega(s)} (f_{k,i})_{u(s)}(E_s)[X_k]_0 \right\}}{F_{u(s)}(E_s)} \tag{2}$$

$$\beta_s = \frac{\sum\limits_{(i,j)} \left\{ K_{j,i} \sum\limits_{k \in \omega(s)} (f_{k,j})_{u(s)-1}(E_s)[X_k]_0 - K_{i,j} \sum\limits_{k \in \omega(s)} (f_{k,i})_{u(s)-1}(E_s)[X_k]_0 \right\} - F_{u(s)-1}(E_s)\alpha_s}{F_{u(s)}(E_s)} \tag{3}$$

$$\gamma_{s,h} = \frac{(-1)^{u(s)-1} \sum\limits_{(i,j)} \left\{ K_{j,i} \sum\limits_{k \in \omega(s)} [X_k]_0 \sum\limits_{q=0}^{u(s)} (f_{k,j})_q(E_s) \lambda_h^{u(s)-q} - K_{i,j} \sum\limits_{k \in \omega(s)} [X_k]_0 \sum\limits_{q=0}^{u(s)} (f_{k,i})_q(E_s) \lambda_h^{u(s)-q} \right\}}{\lambda_h^2 \prod\limits_{\substack{p \in z(s) \\ p \neq h}} (\lambda_p - \lambda_h)} \tag{4}$$

$$[s = 1, 2, \ldots, g; h \in z(s)]$$

Table 1 Example of notation and definitions for Example 2 (Scheme 1) of [1]. The only enzyme species at the onset of the reaction is the free enzyme, E, and the ligand species, Y_s, under study is Y_3 ($s = 3$), *i.e.* P.

Notation/definition	Example 2
n	4
g	3
Initial directed graph	Fig. 1 (a)
Ω	$\{ X_1 \}$
Initial condensation diagram	Fig. 1 (b)
δ	2
classes	$C_1 = \{ X_1, X_2 \}, C_2 = \{ X_3, X_4 \}$
Φ_c	$\{ C_1, C_2 \}$
ρ	$\{ 1, 2 \}$
s (Y_s = P)	3
L	$\{ X_2 \}$
$\boldsymbol{l}$	$\{ 2 \}$
θ	$\{ X_1, X_2 \}$
$\boldsymbol{E}_s$	$\{ C_1 \}$
$\rho(s)$	$\{ 1 \}$
$n(s)$	2
$c(s)$	0
$u(s)$	2
$z(s)$	$\{ 1, 2 \}$
$\omega(s)$	$\{ 1 \}$
$D(\lambda)$	$\begin{vmatrix} K_{1,1} - \lambda & K_{2,1} & 0 & 0 \\ K_{1,2} & K_{2,2} - \lambda & 0 & 0 \\ K_{1,3} & 0 & K_{3,3} - \lambda & K_{4,3} \\ 0 & K_{2,4} & K_{3,4} & K_{4,4} - \lambda \end{vmatrix}$
roots of $T_{E_s}(\lambda)$	$\{ \lambda_1, \lambda_2 \}$

3 Developed Software

Next, we describe the characteristics and use guide for the computer software developed, called SKEE-w2013, in order to fulfil the purposes mentioned in the introduction section. The main characteristics of the algorithm and the public members of the C# developed classes have been moved to the supplementary material section.

3.1 Software Skee-W2013

This informatics tool provides the analytical expressions corresponding to the optimized kinetic equations of those enzyme systems which fit the general model described in [1].

Software SKEE-w2013 has been developed for MS-Windows operating systems in any of their current versions, XP, Vista, 7 and 8. For this task we used the Microsoft Visual Studio 2010 Premium Edition developing package [20] as well as the modern .NET programming technology for C# language [21]. This will facilitate software implementation, design and usability.

The C# implemented classes for this software are described in the supplementary material. For each of these C# classes, a short description of its functionality and the source file content for their public members are indicated.

In the following, we describe the use of this software, which will be supported in section 4, handling different examples of enzyme reaction mechanisms.

3.1.1 Data input

A graphical environment allows us to introduce, in an easy and quick way, the data describing the enzyme system under study, irrespective of its degree of complexity.

Firstly, the user must enter the number, n, of enzyme species involved in the reaction mechanism under study, using the spin buttons located on the upper left of the main window. The software, by default, calls the enzyme species as X1, X2,…, Xn, nevertheless it is possible to assign an alternative notation to each of these enzyme species, e.g. E for X1, ES for X2,…by clicking label *"Inputs:"* on the left side panel and then writing the desired new notation on the displayed form.

Then, in the square matrix, whose size changes according to the n-value, any non-null $K_{i,j}$'s corresponding to the direct connection between enzyme species X_i and X_j must be checked.

Once the non-null $K_{i,j}$'s have been identified, the button *"Set Ki,j's"* is clicked and the program writes, by default, all of these constants $K_{i,j}$'s as $k_{i,j}$'s. If desired, the user can change the expression of any $k_{i,j}$. Any introduced expression for a rate constant is valid, the only restriction being that for pseudofirst order ones the symbol for the ligand species must be between square brackets. Some examples of valid expressions for rate constants are k1[S]0, q4[I]0, k'[M], k+1[S], k2, k'-3, k-1, k'2, k3[I], etc.

The existence of parallel steps between any pair of enzyme species in the reaction mechanisms is also considered. Let us take as an example p parallel steps between X_i and X_j: in these cases, the user must press the "+" button, located on the left side of each $K_{i,j}$, $p-1$ times and then write the expressions for Ki,j(1), Ki,j(2),…, Ki,j(p). For example, if two parallel steps ($p=2$) exist between enzyme species X_4 and X_5, with the expressions of the corresponding rate constants being k5[P] and k+6, the user must indicate this fact, in different rows, as follows:

```
K4,5(1) = k5[P]
K4,5(2) = k+6
```

Parallel steps for a global constant $K_{i,j}$ can be removed by clicking the corresponding button labelled as "−", which deletes them from the previous one.

By default, the program assumes that no ligand species is released in an irreversible reaction step. If some ligand species are irreversibly released then the user must select the radio button *"Yes"* that is associated with the label *"Are there any released Ligands?"*, located in the upper panel of the main window. If the radio button *"Yes"* is selected, the application will show new text fields located on the right side of each

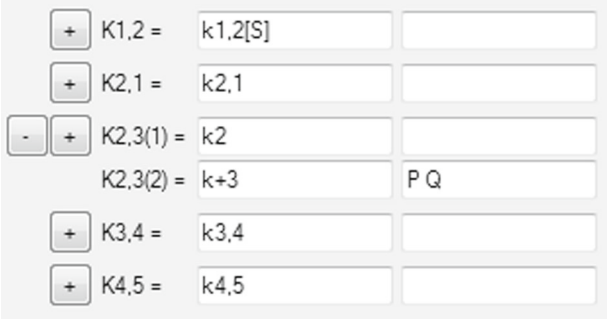


Fig. 2 Screen capture for a hypothetical example of enzyme system consisting in five enzyme species ($X_1, X_2, \ldots, X_5$) and three ligand species (S, P and Q). Substrate S binds to X_1 to give X_2. There are two parallel steps connecting X_2 to X_3, for which the corresponding rate constants are k_2 and k_{+3} and where the ligand species P and Q are irreversibly released in this latter reaction step

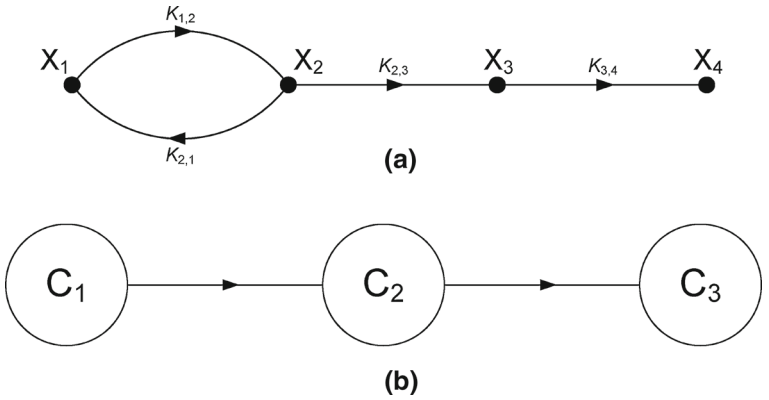
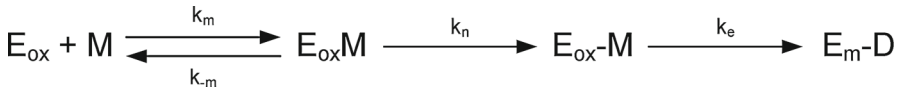


Fig. 3 **a** Directed graph related to the enzymatic reaction scheme shown in Scheme 2. X_1, X_2, X_3 and X_4 denote the compartments that correspond to the enzyme species E_{ox}, $E_{ox}M$, E_{ox}-M and E_m-D, respectively. $K_{1,2}$, $K_{2,1}$, $K_{2,3}$ and $K_{3,4}$ are the fractional transfer coefficients. **b** Condensation diagram corresponding to directed graph of (**a**). Classes are $C_1 = \{X_1, X_2\}$, $C_2 = \{X_3\}$ and $C_3 = \{X_4\}$. The initial class is C_1, C_2 is the transit and C_3 is the final one

constant $K_{i,j}$ described above. In these new text fields, the user can enter the corresponding notation for each irreversible released ligand species, separated by blank spaces, colon or semicolon.

Fig. 2 represents a screen capture for a hypothetical example, consisting of five enzyme species and three ligand species, for which all the cases described above for the input of the rate constants and ligands are contemplated.

Finally, the user must select those enzyme species present at the onset of the reaction, selecting the corresponding boxes in the row labelled as *"Inputs:"* located under the square matrix. Likewise, the species, enzyme and/or ligand whose symbolic time course equation we are interested in must be indicated by selecting the related boxes in the rows labelled as *"Enzyme species:"* and *"Ligand species:"*, for enzyme and ligand species, respectively.



Scheme 2 Enzymatic reaction model proposed by Fujieda *et al.*, [23] where E_{ox} is the *oxi* form of tyrosinase, M is a monophenol and E_m-D complex type *met* of tyrosinase-diphenol.

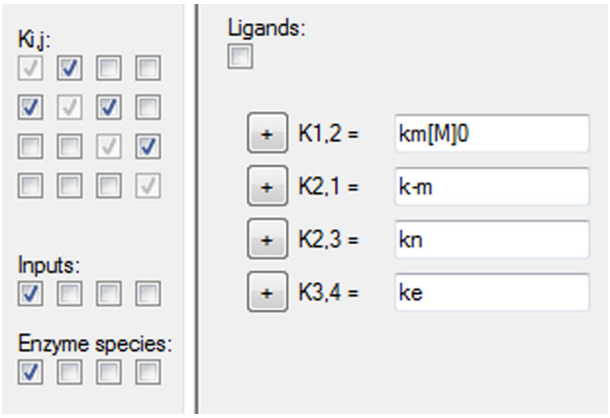


Fig. 4 Screen capture showing data input corresponding to Scheme 2

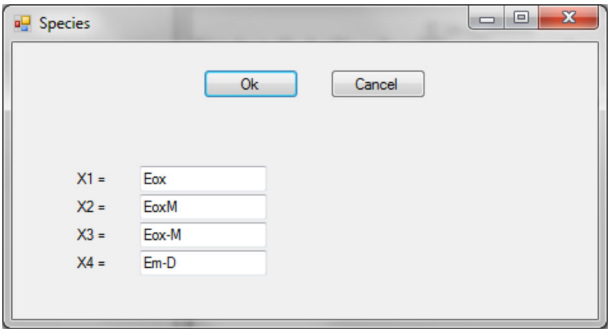


Fig. 5 Dialog form used to assign the expression for each enzyme species of Scheme 2

3.1.2 Results output

Once the data have been entered, as described, the results are obtained after clicking the "Run" button. The results consist of the general optimized symbolic equations, (III-3)-(III-5) for enzyme species and (1)-(4) for ligand species, together with the expressions of coefficients $F_q(E_i)$ $(q = 1, 2, \ldots, u(i))$, $(f_{k,i})_q(E_i)$ $(q = 1, 2, \ldots, u(i))$, $F_q(E_s)$ $(q = 1, 2, \ldots, u(s))$ and $(f_{k,i})_q(E_s)$ $(q = 1, 2, \ldots, u(s))$ as well as the values of all other parameters and indices involved in the equations for each of the species under study.

In order to facilitate reading of the optimized equations, the user can click on the central panel containing these equations to open a new dialog form whereby these

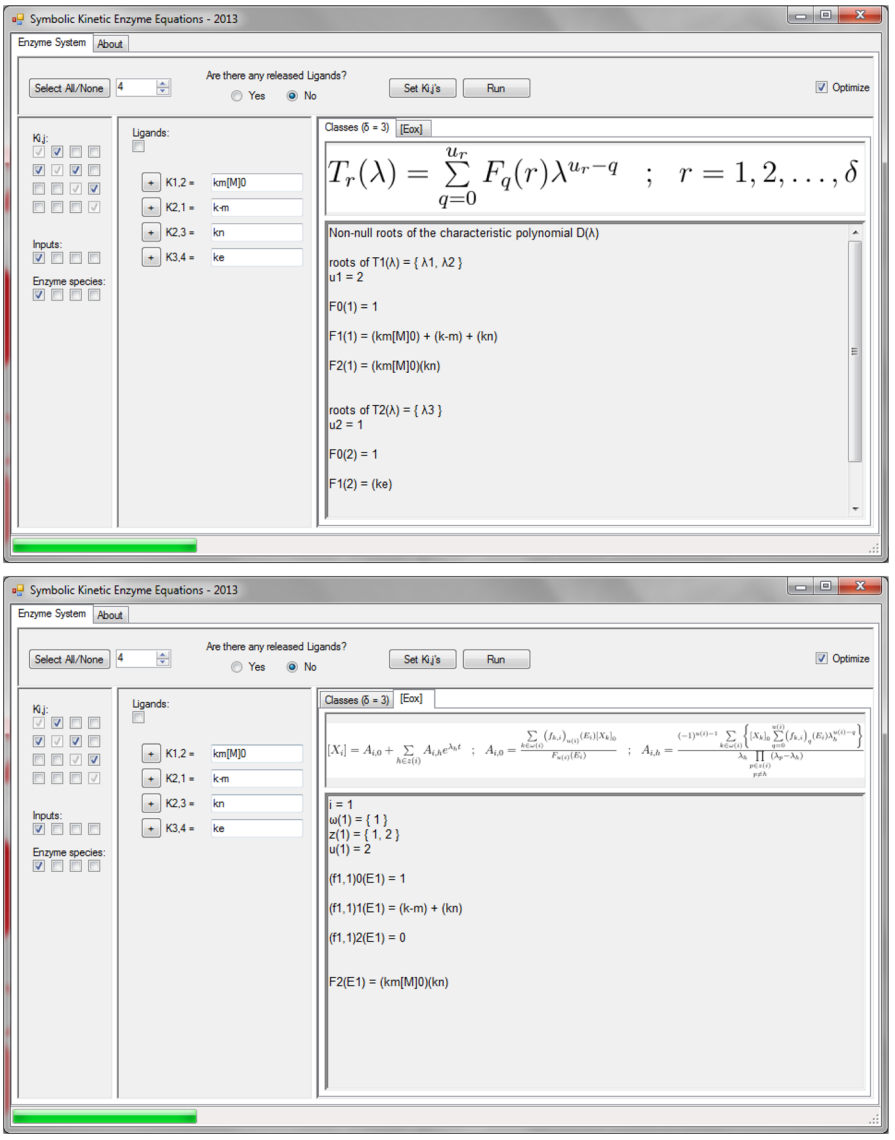


Fig. 6 Screen captures corresponding to: (*Top*) Tab *"Classes"* with the non-null roots of polynomials $T_1(\lambda)$ and $T_2(\lambda)$. (*Bottom*) Tab *"[Eox]"* with $(f_{k,i})_q(E_i)$, $F_{u(i)}(E_i)$ and other necessary quantities to obtain the kinetic equation for $[E_{ox}]$.

equations are shown in greater size. The expressions of the coefficients and the values of different parameters involved in the optimized kinetic equations are given in ASCII format (one textbox for each species).

Let us finally point out that the software also can furnish the non optimized kinetic equations merely unchecking the *"Optimized"* checkbox in the top panel. This option is offered for comparative purposes only.

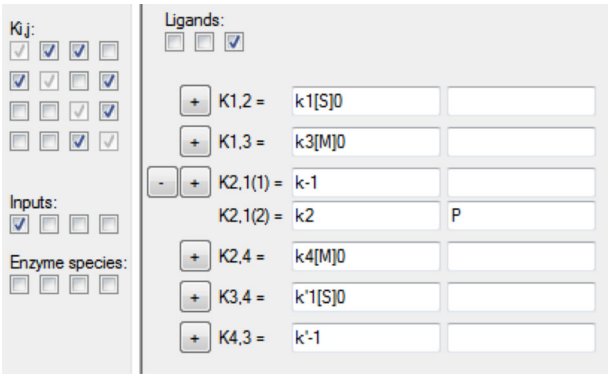


Fig. 7 Screen capture showing data input corresponding to Scheme 1

4 Examples

In this section we obtain, using the software SKEE-w2013, the optimized kinetic equations for the same examples 1 and 2 in [1], which were obtained there manually. Note that, as expected, both results coincide, but here are obtained in a quick and safe way, avoiding human prone errors.

Example 1 will be described with some detail and their extrapolation will allow simplify the corresponding description of the use of the program for example 2, merely throw its screen captures.

Example 1 For easy, we move here its scheme of reaction mechanism as Scheme 2 and the corresponding directed graph and condensation diagram in Fig. 3.

Data input

The data describing this enzyme system are entered as shown in Fig. 4. The expressions for each enzyme species are entered as shown in Fig. 5, after clicking label *"Inputs:"* as described above.

Results output

Once all the data have been entered, the user presses the *"Run"* button for obtaining the desired kinetic equations. In tab *"Classes* ($\delta = 3$)" are indicated the expressions of the polynomial $T_r(\lambda)$ whose roots are involved in these equations. Also, in tab *"[Eox]"* appear the expressions and values for the remaining quantities involved in the corresponding equation. In Fig 6 are shown the screen captures in which these results are given.

Example 2 This example is that indicated in Scheme 1 and Fig. 1. In Figs. 7 and 8, the corresponding screen captures for data input and results output are shown. As indicated in the previous sections, it is possible to obtain the non optimized kinetic equations also. For comparison purposes only, we have included the results for non

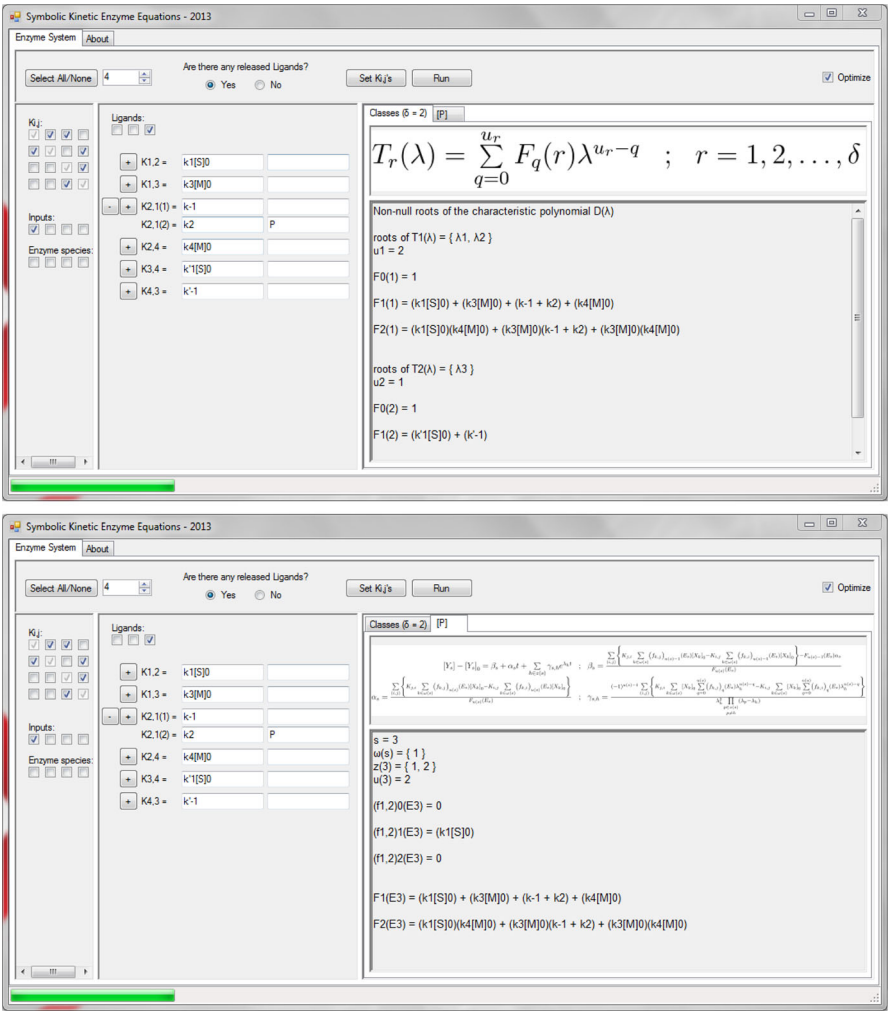


Fig. 8 Screen captures corresponding to: (*Top*) Tab *"Classes"* with the non-null roots of polynomials $T_1(\lambda)$ and $T_2(\lambda)$. (*Bottom*) Tab "[*P*]" with $(f_{k,i})_q(E_s)$, $F_1(E_s)$, $F_2(E_s)$ and other necessary quantities to obtain the kinetic equation for [P]

optimized equations in Fig. 9. Note that the expressions for the involved coefficients are considerably more complex, as expected.

5 Results and discussion

In this paper, we have implemented an informatics tool, called SKEE-w2013, which furnishes the optimized kinetic general equations for the instantaneous concentration of any species involved in a reaction mechanism of enzyme systems fitting the general model described in [1]. Besides the main characteristics detailed below, this software

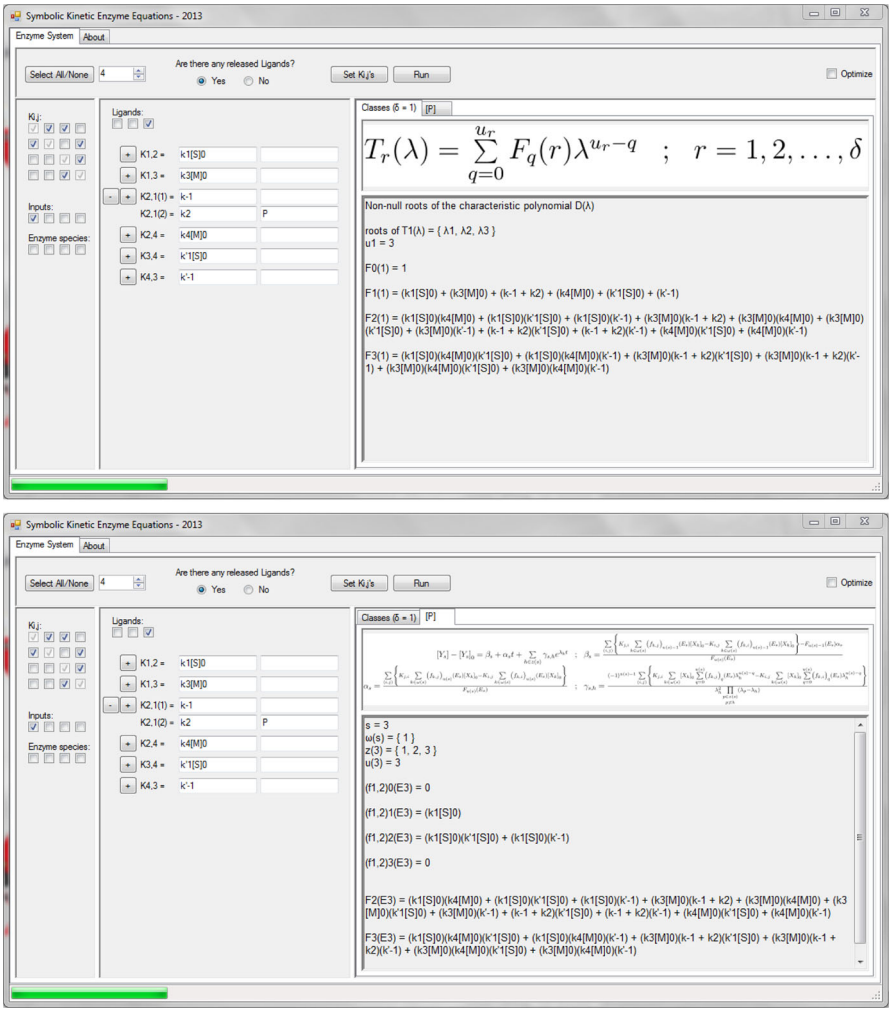


Fig. 9 Screen captures showing the roots, coefficients and parameters of kinetic equation for [P], like in Fig. 8, but using now the non-optimized equations.

presents an important reduction in the CPU computation time with regard to previous programs.

All the examples covered in this paper have been computed under MS-Windows 7 using a compatible PC with Intel Core i5 CPU @ 2.40 GHz and 4 GB of installed memory (RAM). The CPU time in all cases was very short and never took longer than 2 seconds.

Since the number of terms which appear in the expressions of $F_q(E_i)$ and $(f_{k,i})_q(E_i)$ increases quickly when the number of enzyme species and non-null $K_{i,j}$ coefficients increase, we have implemented this software to store the intermediate necessary data in the hard drive rather than in the main RAM memory. This procedure allows its

application to solve enzyme systems of even higher complexity. As already indicated, the software SKEE-w2013 obtains and writes all the terms involved in the analytical expressions. The complexity of the implemented algorithm for calculating these coefficients is n_k^{2q}, where n_k is the number of non-null $K_{i,j}$ in the corresponding class.

In contrast, as indicated for the intermediate data, the final results needed by the symbolic kinetic equations for enzyme and/or ligand species under study are stored in the main RAM memory. This makes it easier for the user to use these results in any other MS-Windows program by mean of the widely known option *"copy and paste"*. This implies that the software can admit character strings of up to 2^{31} bytes, *i.e.* over 2147 million characters, a limit very unlikely to be reached when handling real enzyme systems.

The examples used in this paper are relatively simple and this program's power will be evidenced when it is applied to more complex enzyme systems. With an average desktop PC, like that described before, it should be possible to obtain the symbolic kinetic equations for an enzyme system of up to 60 non-null $K_{i,j}$.

The software presented here, in our opinion, represents an important advance with regard to previous ones.

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